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Determination of fipronil in soil and rice crop at harvest

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ABSTRACT

Residue of fipronil was studied on rice crop following three application of the product at 7 days interval @ 50 and 100 g ai ha^{-1}. The residues were estimated by HPLC equipped with UV detector at 280 nm wavelength. The limit of quantification was 0.001 µg g^{-1} for rice plant, grain and soil. Recoveries of fipronil at 0.01, 0.10 and 0.50 µg g^{-1} fortifications were in the range of 80.00-87.00%, 81.00-89.80%, 87.00-91.40%, and 85.00-89.20% in rice straw, husk, grain and soil, respectively. Residues of fipronil were below maximum residue limit of 0.001 µg g^{-1} at harvest in husk, grain and straw.

Key words: *Fipronil, rice, HPLC, residue, soil*

India is an agricultural country with nearly 62% of the population dependent on agriculture for their livelihood. Rice (*Oryza sativa* L.), is one of the most important food crops in the world and forms the staple diet of 2.7 billion people. Since the introduction of high yielding varieties, distinct changes have occurred in the insect pest complex of rice in India. Pesticides are invaluable inputs for increased agricultural production. The safe use of pesticide depends on its toxicological properties and its distribution and persistence in the environment with consideration of any unusual photoproducts and metabolites that might be formed. Improper usage of pesticides by farmer leads to environmental contamination (Tandon, 2014).

Fipronil [5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*tolyl)-4-trifluoromethyl-sulfinyl pyrazole-3-carbonitrile] is a phenylpyrazole insecticide commonly used in rice, cotton, turf and residential insect. Fipronil represents the second generation of insecticides that disrupts normal nerve function by targeting the γ-aminobutyric acid type A (GABA) receptor system as a noncompetitive blocker in insects (Tomlin, 2000). Fipronil exhibits a high degree of selectivity between insect and mammalian nerve cells (Hainzal et al, 1998). Fipronil is lipophilic and toxic insecticide and is formulated as solid (e.g., insect bait), liquid spray, or as a granular product (e.g., turf application) and these influence its environmental fate (USEPA, 1996). Fipronil degrades to its major metabolites by reduction to sulfide, oxidation to sulfone, hydrolysis to amide, and photolysis to des-sulfinyl (Hainzal and Casida, 1996). Fipronil residues were determined by gas chromatography (GC) with mass spectrometry (MS) or electron capture detector (ECD) using capillary column, with a programmed temperature or by High pressure liquid chromatography (HPLC) with a reversed phase C18 column by some workers (Bobe et al, 1998; Pei et al 2004; Reddy et al 2007; Dutta et al 2008; Liu et al 2008; Wang et al 2013; Kumar et al 2013). Residue estimation of fipronil was not reported in Tarai agro-climatic condition hence, keeping in view of above facts the aim of the study was aimed to analyze fipronil residue in soil and rice plant at harvest of crop in Tarai agro-climatic conditions of Uttarakhand.

MATERIALS AND METHODS

Technical grade, fipronil (96.8% pure) and its formulation 80% WG were obtained from M/s Crystal Crop Protection Pvt. Ltd., Delhi (India). The technical compound were recrystallised, labeled, packed and kept in deep freezer for further use. All the chemicals used during the study were AR / HPLC grade.

Field experiments were conducted at N.E.B. Crop Research Centre, Pantnagar G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand in randomized block design (RBD) with three replications and three treatments during kharif season of 2013-14. The plot size was 5 m × 5m with spacing of 20cm × 15cm. The paddy crop (var. HKR-47) was transplanted and fipronil (80% WG) was applied as a foliar spray at recommended rate (50 g a.i. ha^{-1}) and double recommended rate (100 g a.i. ha^{-1}). There was a control treatment and all the treatments were replicated thrice. The treatment of fipronil 80% WG was done thrice at 7 days time interval for controlling Stem borer, Brown plant hopper, Green leaf hopper Rice leaf hopper, Rice gall midge, Whorl maggot and White backed plant hopper. First spray was done on 42 days after transplanting of crop and the agro-climatic condition during the experimental period was temperature varied from 9.1-33.6^{0}C, Relative humidity 38.3-93.4% and rainfall was 821.4mm.

The samples of paddy grain, straw, husk and soil (0-15 cm depth) from each untreated and treated (fipronil) from each plots were collected at the time of harvest.

Dionex Ultimate 3000 HPLC system with Chromeleon operation consisting of variable wavelength UV detector, binary pump, degassing channels SRD-3200 two vacuum degassing channel, WPS-3000 SL autosampler. The following conditions for HPLC were maintained to get the optimum separation of fipronil. Column: Acclaim 120 C-18 column (250 mm × 2.1 mm × 5µm), Mode: Isocratic, Mobile Phase: Methanol: Water (80:20 v/v), Flow rate: 1 mL min^{-1}, Detector: UV, Wavelength: 280 nm.

A stock solution of 100 µg g^{-1} was prepared by dissolving 1.033 mg of technical grade fipronil in 10 mL of methanol. Different concentrations of fipronil (0.01, 0.05, 0.5, 1.0, 3.0, 5.0 and 10 µg mL^{-1}) was taken for preparation of calibration curve. Twenty micro litre of fipronil of each concentration were injected in triplicate and average detector response in terms of area under the peak was used for preparation of calibration curve.

Extraction and cleanup of samples (rice straw, grain and husk) was done as given below.

Paddy plant (250 g) was taken and ground in motorized blender. Representative samples of paddy plant i.e straw (20 g), was dipped in 100 mL of acetone in conical flask and kept overnight. The extracted samples were re-extracted twice with 50 mL of acetone and filtered. The combined extract was transferred into separatory funnel and diluted with 600 mL of 2% aqueous solution of sodium chloride. This was partioned with 100 mL of dichloromethane. The lower layer of dichloromethane was drained into conical flask through one and half-inch layer (25 gm) of hot anhydrous sodium sulphate supported on a pre-washed glass wool in a funnel. The aqueous layer was re-extracted with 100 mL of dichloromethane and twice with 50 mL of hexane each time and the organic phase were passed through anhydrous sodium sulphate and combined with the contents already obtained. The sodium sulphate was washed with an additional 25 mL of dichloromethane. The combined extracts thus obtained were concentrated to 2 mL under vacuum in a rotary evaporator at a temperature below 38±1°C. The extracts were cleaned up by using silica gel as an adsorbent.

A glass column (10 cm × 1.5 cm i.d.) packed with activated silica gel (2 g) mixed with 0.2 g of charcoal, in between the two small layers of anhydrous sodium sulphate supported on glass wool. The column was pre-washed with hexane, following which the concentrated extract was poured over it. The glass beaker was rinsed with acetone and the extract was transferred to the column. The extract was eluted with a freshly prepared solvent mixture of dichloromethane and acetone (1:1, v/v). The eluate was concentrated to dryness on a rotary evaporator under vacuum and re-suspended to 2 mL of HPLC methanol for HPLC analysis.rushed and powdered grain (20g) and grounded husk (10 g) were extracted and cleanup was done according to the method used for straw as given above.

Pulverised and sieved soil samples (5g) were extracted using acetone: acetonitrile (3:1 v/v) mixture (75 mL) in stoppered flask and shaken on a mechanical shaker for 45 min and the extract was filtered through Whatman No. 1 filter paper. The extraction was done again twice with 50 ml solvent mixture. The extract was pooled, reduced to 2 mL using rotary evaporator at 45±1°C and clean up was done same as done for rice straw.

Recovery studies form soil, grain, husk and

straw were done at 3 different concentrations. i.e. 0.01, 0.10 and 0.50 µg g^{-1}. Shade dried powdered rice grain, straw, husk and soil 5 g each were taken in conical flask and fortified with 1 mL of 0.01, 0.10 and 0.50 µg g^{-1} solution of fipronil (technical grade). The method applied for extraction and cleanup were the same as standardized earlier for straw, grain, husk and soil.

RESULTS AND DISCUSSION

Retention time of fipronil under these conditions was found to be 10.0 min. Calibration curve of fipronil was found to be linear and the determination coefficient (R^2) value was 0.989 respectively. The values of percent recovery of fipronil from fortified samples rice straw, husk, grain and soil varied from 80.00 to 87.00%, 81.00 to 89.80% and 87.00 to 91.40%, 85.00 to 89.20%, respectively (Table 1). The limit of quantification (S/N=10) of fipronil was 0.001 µg g^{-1} for rice plant, grain and soil.

At harvest time residue of fipronil in rice grain, husk, straw and soil were estimated. The result revealed that the residue of fipronil were below the detection limit (< 0.001 µg g^{-1}) for both the treatments in rice grain, husk, straw and soil (Table 2).

The results are in conformation with the studies of Kumar and Singh (2013) who reported that upon application of fipronil at 180 g a.i. ha^{-1}, residues of fipronil and its metabolites were below detectable limit in the paddy plant samples at harvest. According to Wang et al (2013) residues of fipronil and its three metabolites in maize, stem, and soil at harvest time were not detected from two sites and residues were lower than the LOQ (0.002 mg kg^{-1}) and MRL values. Pei *et al* (2004) found that degradation of fipronil was faster in pakchoi (*Brrassica compestris*) and degradation was assisted through oxidation and reduction process. Mohapatra *et al* (2013) found below the quantifiable limit of fipronil and their metabolites (0.01 mg kg^{-1}) at harvest in grape leaves and berries. In Chinese cabbage degradation of fipronil by reduction, oxidization and photodegradation process (Liu *et al.,* 2008). Fipronil in chilli, was dissipated to 0.001 µg g^{-1} within 30 days (Reddy *et al.,* 2007; Kumar *et al.,* 2013).

Table 1. Percent recovery of fipronil from fortified samples of rice grain, husk, straw and soil

Substrate	Amount fortified (µg g^{-1})	Amount recovered (µg g^{-1})±SD	% Recovery	Average % recovery
Rice plant (Straw)	0.01	0.0080±0.003	80.00	84.33
	0.10	0.086±0.007	86.00	
	0.50	0.435±0.010	87.00	
Husk	0.01	0.0081±0.004	81.00	84.33
	0.10	0.0830±0.005	83.00	
	0.50	0.4490±0.012	89.80	
Grain	0.01	0.009±0.002	90.00	89.23
	0.10	0.087±0.010	87.00	
	0.50	0.457±0.010	91.40	
Soil	0.01	0.0085±0.006	85.00	87.10
	0.10	0.0871±0.005	87.10	
	0.50	0.4460±0.015	89.20	

Table 2. Harvest time residues of fipronil in rice grain, husk, straw and soil applied @ 50 g a.i. ha^{-1} and 100 g a.i. ha^{-1}

Sample	Treatment rates	
	50 g a.i. ha^{-1}	100 g a.i. ha^{-1}
Rice grain	B.D.L	B.D.L
Rice husk	B.D.L	B.D.L
Rice straw	B.D.L	B.D.L
Soil	B.D.L	B.D.L

B.D.L (Below Detectable limit) < 0.001 µg g^{-1}

The degradation of fipronil occurred through oxidation, reduction, hydrolysis, photolysis and microbial activities in soils. Under anaerobic and high moisture conditions fipronil breakdown is rapid in soils. Oxidation-reduction process lead to breakdown fipronil into its consecutive metabolites i.e. sulfone and sulphide, hydrolysis of the nitrile group of fipronil to an amide group (USEPA, 1996).

Since the residue of fipronil were below detection limit in both edible and non edible part of rice its efficient usage can be inferred as safe from

consumption point for human beings, animals and environmental point of view.

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